MAYO CLINIC LABORATORIES

# Test Definition: SPEP

Electrophoresis, Protein, Serum

## Overview

### Useful For

Screening patients with suspected monoclonal gammopathies

Diagnosis of monoclonal gammopathies, when used in conjunction with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) and free light chain analysis

### Profile Information

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| TPE | Total Protein | Yes, (Order TP) | Yes |
| SPE | Protein Electrophoresis | No | Yes |

### Reflex Tests

| Test Id | Reporting Name | Available Separately | Always Performed |
|---|---|---|---|
| IFXED | Immunofixation Delta and Epsilon, S | Yes | No |
| MPTS | M-protein Isotype MALDI-TOF MS, S | Yes, (Order MALD) | No |

### Testing Algorithm

This test includes total protein and serum protein electrophoresis.

If a discrete electrophoresis band is identified, the laboratory will evaluate the serum protein electrophoresis and, if necessary, perform M-protein isotype at an additional charge.

If a light chain is identified without a corresponding heavy chain during initial testing, immunofixation with IgD and IgE antisera will be performed at an additional charge.

The following algorithms are available:
-Amyloidosis: Laboratory Approach to Diagnosis
-Multiple Myeloma: Laboratory Screening

### Special Instructions

- Amyloidosis: Laboratory Approach to Diagnosis
- Multiple Myeloma: Laboratory Screening

### Method Name

TPE: Colorimetric, Biuret
SPE: Agarose Gel Electrophoresis

### NY State Available
Yes

## Specimen

### Specimen Type
Serum

### Ordering Guidance
Protein electrophoresis alone is not considered an adequate screen for monoclonal gammopathies. When screening a patient or establishing a first-time diagnosis for a monoclonal gammopathy, consider ordering DMOGA / Monoclonal Gammopathy, Diagnostic, Serum instead, which includes free light chain analysis and isotyping by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS).

If free light chain testing has already been performed locally, PEISO / Protein Electrophoresis and Isotype, Serum may be ordered instead of DMOGA / Monoclonal Gammopathy, Diagnostic, Serum for a first-time diagnosis.

For monitoring patients with a diagnosis of monoclonal gammopathy, order TMOGA / Monoclonal Gammopathy, Monitoring, Serum.

### Necessary Information
Indicate if multiple myeloma is suspected.

### Specimen Required
**Patient Preparation:** Fasting (12 hour) preferred but not required
**Collection Container/Tube:**
**Preferred:** Serum gel
**Acceptable:** Red top
**Submission Container/Tube:** Plastic vial
**Specimen Volume:** 1 mL
**Collection Instructions:** Centrifuge and aliquot serum into plastic vial.

### Forms
If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-General Request (T239)
-Renal Diagnostics Test Request (T830)

### Specimen Minimum Volume
0.5 mL

### Reject Due To

| | |
|---|---|
| Gross hemolysis | OK |

| | |
|---|---|
| Gross lipemia | OK |
| Gross icterus | OK |

### Specimen Stability Information

| Specimen Type | Temperature | Time | Special Container |
|---|---|---|---|
| Serum | Refrigerated (preferred) | 14 days | |
| | Frozen | 14 days | |
| | Ambient | 7 days | |

## Clinical & Interpretive

### Clinical Information

This profile includes both total protein and protein electrophoresis. The serum proteins can be grouped into 5 fractions by protein electrophoresis:
-Albumin, which represents almost two-thirds of the total serum protein
-Alpha-1, composed primarily of alpha-1-antitrypsin (A1AT), an alpha-1-acid glycoprotein
-Alpha-2, composed primarily of alpha-2-macroglobulin and haptoglobin
-Beta, composed primarily of transferrin and C3
-Gamma, composed primarily of immunoglobulins

The concentration of these fractions and the electrophoretic pattern may be characteristic of diseases such as monoclonal gammopathies, A1AT deficiency disease, nephrotic syndrome, and inflammatory processes associated with infection, liver disease, and autoimmune diseases.

### Reference Values

> or =1 year: 6.3-7.9 g/dL
Reference values have not been established for patients that are younger than 12 months of age.

PROTEIN ELECTROPHORESIS
Albumin: 3.4-4.7 g/dL
Alpha-1-globulin: 0.1-0.3 g/dL
Alpha-2-globulin: 0.6-1.0 g/dL
Beta-globulin: 0.7-1.2 g/dL
Gamma-globulin: 0.6-1.6 g/dL
An interpretive comment is provided with the report.
Reference values have not been established for patients that are younger than 16 years of age.

### Interpretation

Monoclonal Gammopathies:
A characteristic monoclonal band (M-spike) is often found on serum protein electrophoresis (SPE) in the gamma-globulin region and, more rarely, in the beta or alpha-2 regions. The finding of a M-spike, restricted migration, or hypogammaglobulinemic SPE pattern is suggestive of a possible monoclonal protein and should be confirmed by immunoaffinity-purification matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF

MS) to identify any immunoglobulin heavy or light chains. A MPSU / Monoclonal Protein Study, 24 Hour, Urine is suggested for first-time M-spike patients to assess for renal disease that can be associated with an M-spike.
-A monoclonal IgG or IgA greater than 3 g/dL is consistent with multiple myeloma (MM).
-A monoclonal IgG or IgA less than 3 g/dL may be consistent with monoclonal gammopathy of undetermined significance, primary systemic amyloidosis, early or treated myeloma, as well as a number of other monoclonal gammopathies.
-A monoclonal IgM greater than 3 g/dL is consistent with macroglobulinemia.
-The initial identification of a serum M-spike greater than 1.5 g/dL on SPE should be followed by MPSU / Monoclonal Protein Study, 24 Hour, Urine.
-The initial identification of an IgM, IgA, or IgG M-spike greater than 4 g/dL, greater than 5 g/dL, and greater than 6 g/dL, respectively, should be followed by SVISC / Viscosity, Serum.

After the initial identification of an M-spike, quantitation of the M-spike on follow-up SPE can be used to monitor the monoclonal gammopathy. However, if the monoclonal protein falls within the beta region (most commonly an IgA or an IgM) quantitative immunoglobulin levels may be more a useful tool to follow the monoclonal protein level than SPE. A decrease or increase of the M-spike that is greater than 0.5 g/dL is considered a significant change.

Patients suspected of having a monoclonal gammopathy may have normal serum SPE patterns. Approximately 11% of patients with MM have a completely normal serum SPE, with the monoclonal protein only identified by MALDI-TOF MS. Approximately 8% of MM patients have hypogammaglobulinemia without a quantifiable M-spike on SPE but identified by MALDI-TOF MS. Accordingly, a normal serum SPE does not rule out the disease and should not be used to screen for the disorder. The DMOGA / Monoclonal Gammopathy, Diagnostic, Serum, which includes MALDI-TOF MS, and serum free light chains, conforms to the International Myeloma Working Group guidelines for screening and should be performed if there is clinical suspicion.

Other Abnormal SPE Findings:
-A qualitatively normal but elevated gamma fraction (polyclonal hypergammaglobulinemia) is consistent with infection, liver disease, or autoimmune disease.
-A depressed gamma fraction (hypogammaglobulinemia) is consistent with immune deficiency and can also be associated with primary amyloidosis or nephrotic syndrome.
-A decreased albumin (<2 g/dL), increased alpha-2 fraction (>1.1 g/dL), and decreased gamma fraction (<1 g/dL) is consistent with nephrotic syndrome, and when seen in an adult older than 40 years, should be followed by MPSU / Monoclonal Protein Study, 24 Hour, Urine.
-In the hereditary deficiency of a protein (eg, agammaglobulinemia, alpha-1-antitrypsin [A1AT] deficiency, hypoalbuminemia), the affected fraction is faint or absent.
-An absent alpha-1 fraction is consistent with A1AT deficiency disease and should be followed by a quantitative A1AT assay (AAT / Alpha-1- Antitrypsin, Serum).

### Cautions

Very large IgG M-spikes (>4 g/dL) may saturate the protein stain. In these situations, quantitative IgG assays (IGG / Immunoglobulin G [IgG], Serum) should be performed to accurately determine M-spike concentrations to monitor disease progression or response to therapy.

Fibrinogen will migrate as a distinct band in the beta-gamma fraction. Serum specimens from new patients with a beta-gamma band are to be treated with thrombin to ensure complete conversion of fibrinogen.

Hemolysis may augment the beta fraction.

Penicillin may split the albumin band.

Radiographic agents may produce an uninterpretable pattern.

### Clinical Reference

1. Kyle RA, Katzmann JA, Lust JA, Dispenzieri A: Clinical indications and applications of electrophoresis and immunofixation. In: Rose NR, Hamilton RG, Detrick B, eds. Manual of Clinical Laboratory Immunology. 6th ed. ASM Press. 2002:66-70
2. Mills JR, Kohlhagen MC, Dasari S, et al: Comprehensive assessment of M-Proteins using nanobody enrichment coupled to MALDI-TOF mass spectrometry. Clin Chem. 2016 Oct;62(10):1334-1344
3. Milani P, Murray DL, Barnidge DR, et al: The utility of MASS-FIX to detect and monitor monoclonal proteins in the clinic, Am J Hematol. 2017 Aug;92(8):772-779. doi: 10.1002/ajh.24772

## Performance

### Method Description

Electrophoresis:
Serum proteins are separated in an electric field according to their size, shape, and electric charge. The separation is performed on agarose gels. The proteins are visualized by staining with acid blue, and the intensity of staining is quantitated by densitometry. Multiplying by the serum total protein (Coomasie blue) converts the percentage of protein in each fraction into serum concentration.(Instruction manual: Helena SPIFE Touch. Helena Laboratories, Corp; 11/2016; package insert: Helena SPIFE Touch SPE Pro 277. Helena Laboratories, Corp; 06/2018)

Immunofixation
Immunofixation is performed with Sebia reagent sets that are specific for delta and epsilon immunoglobulin heavy chains and kappa and lambda light chains. Immunofixation electrophoresis is performed in four stages: 1) separation of proteins by electrophoresis on an agarose gel; 2) immunofixation (immunoprecipitation) and fixation of the electrophoresed proteins;3) removal of unprecipitated soluble proteins by blotting and washing;, and 4) staining of the precipitated proteins for visualization.(Package insert: Sebia HYDRAGEL 1, 2, 4 and 9 IF kit. Sebia Inc; 07/2020)

M-protein Isotype:
M-protein isotype by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is performed with immunoaffinity purification followed by MALDI-TOF MS analysis. For the immunoaffinity purification, patient serum is applied to 5 separate immunoaffinity resins (CaptureSelect, Life Sciences) specific to immunoglobulin G, A, M, K, and L. Unbound protein is washed away and the isolated immunoglobulins are broken down into their reduced to separate the heavy and light chains subunits to be analyzed via MALDI-TOF mass spectrometry. The 5 separate spectra from each patient immunopurification are overlaid and investigated for an overabundance of immunoglobulin and immunoglobulin light chain.(Milani P, Murray DL, Barnidge DR, et al: The utility of MASS-FIX to detect and monitor monoclonal proteins in the clinic. Am J Hematol. 2017;92(8):772-779. doi: 10.1002/ajh.24772)

### PDF Report

No

### Day(s) Performed
Monday through Friday

### Report Available
2 to 5 days

### Specimen Retention Time
14 days

### Performing Laboratory Location
Rochester

## Fees & Codes

### Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

### Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

### CPT Code Information
84155
84165
0077U (if appropriate)
86334 (if appropriate)

### LOINC® Information

| Test ID | Test Order Name | Order LOINC® Value |
|---|---|---|
| SPEP | Electrophoresis, Protein, S | 24351-9 |

| Result ID | Test Result Name | Result LOINC® Value |
|---|---|---|
| TPE | Total Protein | 2885-2 |
| 602837 | Albumin | 2862-1 |
| 602838 | Alpha-1 Globulin | 2865-4 |
| 602839 | Alpha-2 Globulin | 2868-8 |
| 602840 | Beta-Globulin | 2871-2 |
| 602841 | Gamma-Globulin | 2874-6 |
| 602842 | A/G Ratio | 44429-9 |

| | | |
|---|---|---|
| 602843 | M spike | 51435-6 |
| 602844 | M spike | 35559-4 |
| 602836 | Impression | 49296-7 |